

Influence of Supplementing Pasture-Based Primiparous Holstein-Friesian Dairy Cows with Crude Degummed Canola Oil on Milk Fatty Acid Composition

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Abstract: The quest for alternative sources of healthy nutrients that facilitate the modification of milk without compromising drinking quality is a continuous research endeavour. The objective of the study was to quantify the milk fatty acid composition of pasture-based primiparous Holstein-Friesian dairy cows supplemented with crude degummed canola oil (CDCO) with a view to improving the milk quality for beneficial health effects. This study tested the hypothesis that incremental supplementation of grazing primiparous Holstein-Friesian cows with CDCO will alter milk fatty acid composition towards increased total monounsaturates. Comparisons were made between unsupplemented grazing dairy cows and their peers on dietary supplements containing low (25ml/Kg DM), medium (35ml/Kg DM) or high levels (50ml/kg DM) of CDCO in addition to *ad libitum* grazing access to pasture. There was no significant effect ($p>0.05$) of CDCO supplementation for eight weeks on the proportions of total polyunsaturated fatty acids (tPUFA), omega-3 (ω -3) and omega-6 (ω -6) fatty acids in milk. However, significant impacts of CDCO were observed on the proportions of 18:1 ω 9c, 18:1 ω 7t, total saturated (tSFA) and total monounsaturated (tMUFA) fatty acids ($p<0.005$), with a significant increase in the tMUFA/tSFA ratio in cows consuming CDCO. It was concluded that incremental levels of CDCO supplementation can modify the fatty acid composition of milk towards increased monounsaturates without any negative impact on grazing primiparous cows.

Keywords: Monounsaturated Fatty Acids, Polyunsaturated Fatty Acids, Saturated Fatty Acids, omega-3, omega-6.

INTRODUCTION

The demand for milk and other dairy products has slightly increased in Australia, with the consumption of drinking milk per capita rising from 104.4 liters in 2010/11 to 106.2 liters in 2011/12, respectively [1]. The primary focus of dairy farmers is to increase milk production with adequate fat and protein compositions because of the associated economic benefits of milk solids. In response to health concerns about coronary heart disease, obesity and arteriosclerosis, research interests in modifying milk fatty acid composition toward less saturated medium-chain ($\leq C12$) fatty acids and more long-chain ($\geq C18$) polyunsaturated fatty acids (PUFA) are on the increase. The simplest way of altering milk fat composition is to supplement the diets

of cows with unsaturated lipids [2,3]. Milk fat composition is changed more by the amount and composition of dietary fat than any other dietary component, and several studies [4-6] have been published on the response of milk fat composition to dietary lipid supplements in dairy cows. However, in Tasmania's pasture-based dairy production system, dietary supplementation of lactating cows with fat is not a common nutritional management practice, mainly because of its unknown impacts on milk fatty acid composition and other lactation traits. Previous fat studies in other dairy systems have reported the effects of fat supplements on milk fatty acid profiles [2, 3, 6]. Dietary fat supplementation of dairy cows in pasture-based production systems has been targeted toward enhancing the proportions of ω -3 and ω -6 fatty acids at the expense of SFA to achieve desirable human health benefits [7-9]. However, the beneficial health effect of fat supplements can be countered by the concurrent production of *trans*-monounsaturated fatty acids (MUFA) known to be associated with cholesterol [10, 11]. However, published studies in Australia

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investigating the impact of dietary fat supplementation using crude degummed canola oil (CDCO) on milk fatty acid profiles of pasture-based primiparous cows are at best, scanty or non-existent, hence the need for this study to fill in the knowledge gap.

Canola oil products are readily available in Australia, and represent an excellent source of dietary fat, especially oleic acid [12, 13]. However, extensive rumen biohydrogenation of canola can lead to the formation of *trans*-MUFA, an intermediate carbon-chain group of fatty acids [14]. Therefore, robust knowledge is required about the impact of supplementing lactating cows with CDCO on milk fatty acid composition. Furthermore, contrasting reports on the effect of canola supplementation on milk fatty acids abound in the published literature, but there is a dearth of rigorously peer-reviewed information on the use of CDCO as a supplement in pasture-based dairy production systems. However, studies conducted elsewhere using soybean and linseed oil reported an increase in the proportion of PUFA (C18:2 *cis*-9,12 and C18:3 *cis*-9,12,15), whereas feeding cows with rapeseed oil decreased the proportion of MUFA (C18:1 *cis*-9) in milk fat [15]. Therefore in achieving this paper's objective, it was hypothesized that incremental supplementation of grazing primiparous Holstein-Friesian cows with CDCO will alter milk fatty acid composition towards increased total monounsaturates.

MATERIALS AND METHODS

All experimental procedures were in accordance with the University of Tasmania (UTAS) Animal Ethics Committee guidelines, the 1993 Tasmania Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Site and Climatic Conditions

The experiment was carried out in spring from September to November 2012 at the Dairy Research Centre of the Tasmanian Institute of Agriculture in Somerset, North-West of Tasmania, Australia, when the annual rainfall and humidity were approximately 2500mm and 60%, respectively. Tasmania is Australia's smallest state with a land size of 68,000 square kilometres and located within the cool, temperate, climatic zone at latitude 42° South and longitude 147° East characterized by four distinct seasons (winter, autumn, spring and summer).

Animals and Treatments

Body condition score (BCS) of the cows was visually assessed on a scale of 1 to 8 [16, 17]. A total of 20 primiparous, spring-calving, and purebred Holstein-Friesian cows (average live-weight of 400 ± 40 Kg, BCS 4 ± 1 and 40 ± 8 DIM) were randomly allocated into 1 of 4 treatments of CDCO (25ml/ Kg DM, 35ml/ Kg DM and 50ml/ Kg DM) and the control (no CDCO 0ml/ Kg DM). All experimental cows ($n=5$ per treatment group) were placed under the same grazing management and rotated in electric-fenced paddocks. The Control group of cows were offered wheat-based pellets with no CDCO and grazed on the same pastures comprising a mixture of ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*), and white clover (*Trifolium repens*). Water was offered *ad libitum*. Cows in all the other treatment groups also grazed the same pastures as the Control treatment cows but were offered CDCO plus wheat-based pellets at the rate of 50ml/Kg DM (High), 35ml/Kg DM (Medium) and 25ml/Kg DM (Low) level of supplementation. The current level of CDCO was based on 7% total fat in the diet of grazing cows [18]. Supplements were offered to cows in two splits of 3kg each during morning and evening milking sessions at 05:00 h and 15:00 h, respectively, hence each cow received 6 kg of the pelleted supplement daily for eight weeks after two weeks of adjustment. There were no left-over feed residuals from any of the group of cows. The chemical and fatty acid compositions of the treatment, control and basal feeds are presented in Tables 1 and 2.

Feed Chemical Composition and Analysis

Dry matter (DM) content of the basal and experimental diets was determined by drying the samples to a constant temperature at 65°C in a fan-forced oven, finely ground to pass through a 2mm sieve using a Laboratory Mill (Thomas Model 4 Wiley® Mill; Thomas Scientific), and further drying at 105°C for 24h. The DM was computed as the difference between the initial and final weights of the samples. Ash content was determined by combusting the samples in a furnace at 600°C for 8 hours. Neutral detergent (NDF) and acid detergent (ADF) fiber contents were measured using an Ankom fiber analyzer ANKOM220; ANKOM Technology, USA. [19]. Total nitrogen was determined using a Thermo Finnigan EA 1112 Series Flash Elemental Analyzer [20] and the values multiplied

Table 1: Chemical Composition of Experimental and Basal Feeds

^c Chemical composition	^a Feed components			^b Units
	Treatment Feed (high canola oil)	Control Feed (No canola oil)	Basal Feed (Pasture)	
MC	8.2	9.1	55.0	g/100g DM
DM	91.8	90.9	94.5	g/100g DM
ADF	8.0	9.0	27.7	g/100g DM
NDF	20.0	21.1	45.9	g/100g DM
EE	6.2	2.1	3.0	g/100g DM
Ash	9.7	8.9	9.3	g/100g DM
NFC	52.8	59.0	23.9	g/100g DM
CP	12.7	10.4	21.0	g/100g DM
ME	4083.3	4065.7	3999.2	kJ/100g DM

^{a-b} All feeds were analyzed based on a dry weight basis; ^cMoisture content (MC), Dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), non-fibrous carbohydrate (NFC), ether extract (EE), crude protein (CP) and metabolisable energy (ME).

by 6.25 to give the crude protein (CP) percentage. Ether extract (EE) was determined using an Ankom fat/oil extractor (ANKOM^{XT15}; ANKOM Technology, USA). Metabolisable energy (ME) was calculated as per Weiss [21].

Milk Sample Collection

Weekly milk samples were bulked from daily consecutive milkings at 05:00h and 15:00h for 8 weeks (2,240 samples in total). Representative aliquots of fresh milk samples from each cow were collected using the Milking Point Controller (MPC 680) fitted to the De Laval herringbone milking machine into labelled plastic vials containing bronopol blue milk preservative and stored at -20°C until further analysis [22]. No experimental cow suffered mastitis before, during or after the feeding trial period.

Milk Fatty Acid Analysis

The milk samples were analysed using the GLC method applied by the Commonwealth Scientific and Industrial Research Organization (CSIRO) Food Futures Flagship's Omega-3 Research Group, Marine and Atmospheric Research, Hobart, Tasmania, Australia, following direct methylation according to International Organization for Standardization (ISO) procedures. The procedure was as follows: Approximately 0.5g of milk was freeze-dried and 0.05mg of feed samples were weighed in duplicates into clean, 10ml screw-top methylation tubes and a freshly made solution of trans-esterification reaction mix (methanol:hydrochloric acid:chloroform (10:1:1 v/v/v, 3ml) was added. Aliquots of milk were suspended in the trans-esterification solution and vortexed before trans-esterification at 80°C for two hours. Each test

tube was cooled for five minutes before 1ml of MilliQ water was added and the fatty acid methyl esters (FAME) were extracted using 3 x 2ml of hexane:dichloromethane at a ratio of 4:1 v/v. Extracts from the methylation tubes were pipetted into vials, diluted with a known concentration of 19:0 FAME contained in chloroform as the internal injection standard and were ready for gas chromatographic analysis. Chloroform was added to two vial tubes to form the blank controls for milk and feed samples. An Agilent Technologies 7890B GC equipped with a 15m x 0.11mm internal diameter cross-linked Equity-1 (0.1µm film thickness) fused-silica capillary column, a split/splitless injector, a 7683B series autosampler and flame ionization detector [23] was used to analyze the FAME. Quantification of recorded peak areas was carried out using the software package Agilent Technologies Chemstation (Palo Alto, CA, USA). FAME identity was confirmed by a GCQ (Thermoquest, USA) GC-mass spectrometer (GC-MS)', fitted with an on-column injector and an HP-5 cross-linked methyl silicone fused silica capillary column (50 m×0.32mm i.d.) of similar polarity to that described above. Quantification of recorded peak areas was carried out using the software package Millennium 32 v3.05.01 (Waters Corporation, USA). FAME identity was confirmed by an MD 800 (Fissions, UK) or GCQ (Thermoquest, USA) GC-mass spectrometers (GC-MS) [23]. Quantified peaks were exported into an excel file, converted to total fatty acid percentages and subjected to statistical analysis.

Statistical Analysis

Initially, summary statistics by level and week of supplementation were computed to obtain means, standard deviations, standard error, minimum and

Table 2: Fatty Acids (% of Total Fatty Acids) in Basal and Experimental Feeds

^a Fatty acid (%)	Feed Component		
	Control (No canola oil) %	Treatment (high canola oil) %	Basal (Pasture) %
12:0	0.00	0.00	0.05
14:0	0.10	0.09	0.10
15:0	0.20	0.13	0.20
16:1	0.00	0.00	1.00
16:0	32.10	26.10	10.00
17:0	0.20	0.18	0.10
18:3 ω 6	0.00	0.03	0.00
18:4 ω 3	0.00	0.00	0.90
18:2 ω 6 LA	17.70	6.86	9.10
18:3 ω 3 ALA	1.60	0.48	64.30
18:1 ω 9c	16.50	41.90	4.40
18:1 ω 7t	0.2	0.1	0.2
18:0	3.80	3.83	2.20
18:2CLAa	0.00	0.37	0.00
18:2CLAb	0.10	1.11	0.00
19:0	0.90	3.47	0.10
20:4 ω 6 ARA	0.00	0.01	0.00
20:5 ω 3 EPA	11.80	0.20	0.10
20:3 ω 6	0.40	1.82	0.80
20:4 ω 3 ETA	0.40	0.22	0.10
20:2 ω 6	1.40	1.45	0.00
20:0	0.80	1.38	0.40
22:5 ω 6	0.30	0.04	0.10
22:6 ω 3 DHA	0.20	0.03	0.00
22:4 ω 6	0.20	0.00	0.00
22:5 ω 3 DPA	0.90	0.00	0.00
22:0	1.80	1.86	1.50
24:0	1.10	1.30	0.90
Σ SFA	41.20	38.64	16.45
Σ MUFA	23.30	48.74	8.00
Σ PUFA	35.00	12.62	75.40
ω -3 PUFA	14.90	0.93	65.40
ω -6 PUFA	20.10	10.24	10.10
ω -3 LC-PUFA	13.30	0.45	0.20

^a Σ SFA is the sum of 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 22:0, 24:0; Σ MUFA is the sum of 14:1 ω -5c, 15:1 ω -6c, 16:1 ω -7c, 16:1 ω -7t, 16:1 ω -5c, 16:1, 17:1 ω -8+a17:0, 17:1 ω -6c, 18:1 ω -9c, 18:1 ω -7c, 18:1 ω -7t, 18:1 ω -5c, 18:1a, 18:1b, 20:1 ω -11c, 20:1 ω -9c, 20:1 ω -7c, 20:1 ω -5c, 22:1 ω -11c, 22:1 ω -9c, 22:1 ω -7c, 24:1 ω -11c, 24:1 ω -9c, 24:1 ω -7c; Σ PUFA is the sum of 18:3 ω -6, 18:4 ω -3, 18:2 ω -6, 18:3 ω -3, 18:2CLAa, 18:2CLAb, 20:4 ω -6, 20:5 ω -3, 20:3 ω -6, 20:4 ω -3, 20:2 ω -6, 22:5 ω -6, 22:6 ω -3, 22:4 ω -6, 22:5 ω -3; Σ ω -3 LC-PUFA is the sum of 20:5 ω -3, 20:4 ω -3, 22:6 ω -3, 22:5 ω -3; Σ ω -3 PUFA is the sum of 18:4 ω -3, 18:3 ω -3, 20:4 ω -3, 20:5 ω -3, 22:6 ω -3, 22:5 ω -3; Σ ω -6 is the sum of 15:1 ω -6, 17:1 ω -6, 18:2 ω -6, 18:3 ω -6, 20:4 ω -6, 20:3 ω -6, 20:2 ω -6, 22:5 ω -6, 22:4 ω -6. Σ SFA= total saturated fatty acids, Σ MUFA= total monounsaturated fatty acids, Σ PUFA= total polyunsaturated fatty acids, ω -3 FA= total omega-3 fatty acids, ω -6 FA=total omega-6 fatty acids, ω -3 LC-FA=total omega-3 long chain fatty acids.

Table 3: Mean Fatty Acid Concentration (\pm SE) (% Total FA) of Milk Samples by Level of Supplementation with CDCO

^d Fatty acid (%)	^e Treatment group				RMSE	P value	
	Control	Low	Medium	High		TRT	TRT*WK
14:0	14.36 \pm 0.3 ^b	15.37 \pm 0.5 ^a	13.62 \pm 0.5 ^c	13.26 \pm 0.3 ^c	1.94	***	NS
15:0	2.00 \pm 0.1	1.53 \pm 0.1	1.45 \pm 0.1	1.31 \pm 0.1	0.37	NS	NS
16:1	0.36 \pm 0.0	0.37 \pm 0.0	0.31 \pm 0.0	0.29 \pm 0.0	0.07	NS	NS
16:0	34.01 \pm 1.1 ^a	31.97 \pm 1.7 ^a	28.48 \pm 1.2 ^b	27.49 \pm 0.9 ^b	0.69	**	NS
17:0	0.69 \pm 0.0	0.57 \pm 0.0	0.59 \pm 0.0	0.56 \pm 0.0	0.10	NS	NS
18:3 ω 6	0.03 \pm 0.0	0.03 \pm 0.0	0.02 \pm 0.0	0.02 \pm 0.0	0.01	NS	NS
18:4 ω 3	0.03 \pm 0.0	0.03 \pm 0.0	0.05 \pm 0.0	0.06 \pm 0.0	0.04	NS	NS
18:2 ω 6	2.13 \pm 0.2	2.11 \pm 0.16	2.26 \pm 0.1	2.39 \pm 0.1	0.69	NS	NS
18:3 ω 3	0.83 \pm 0.1	0.85 \pm 0.1	0.95 \pm 0.1	0.97 \pm 0.1	0.53	NS	NS
18:1 ω 9c	16.09 \pm 0.6 ^b	16.38 \pm 0.5 ^b	19.64 \pm 0.6 ^a	19.58 \pm 0.6 ^a	2.84	***	NS
18:1 ω 7t	5.01 \pm 0.4 ^b	5.69 \pm 0.5 ^b	6.01 \pm 0.3 ^b	7.49 \pm 0.5 ^a	2.14	***	NS
18:0	7.16 \pm 0.5 ^b	7.60 \pm 0.5 ^b	9.28 \pm 0.5 ^a	8.85 \pm 0.5 ^a	2.09	***	NS
18:2CLAa	1.13 \pm 0.1	1.16 \pm 0.1	1.26 \pm 0.1	1.40 \pm 0.1	0.31	**	NS
18:2CLAb	0.25 \pm 0.0	0.24 \pm 0.0	0.26 \pm 0.0	0.29 \pm 0.0	0.08	*	NS
19:0	0.05 \pm 0.0	0.05 \pm 0.0	0.05 \pm 0.0	0.06 \pm 0.0	0.02	*	NS
20:5 ω 3 EPA	0.09 \pm 0.0	0.09 \pm 0.0	0.08 \pm 0.0	0.08 \pm 0.0	0.02	NS	NS
20:3 ω 6	0.08 \pm 0.0	0.08 \pm 0.0	0.07 \pm 0.0	0.07 \pm 0.0	0.02	*	NS
20:4 ω 3	0.04 \pm 0.0	0.04 \pm 0.0	0.04 \pm 0.0	0.04 \pm 0.0	0.02	NS	NS
20:2 ω 6	0.03 \pm 0.0	0.04 \pm 0.0	0.04 \pm 0.0	0.08 \pm 0.0	0.03	***	NS
20:0	0.09 \pm 0.0	0.10 \pm 0.0	0.11 \pm 0.0	0.13 \pm 0.0	0.04	***	NS
22:6 ω 3	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.00 \pm 0.0	0.01	NS	NS
22:4 ω 6	0.00 \pm 0.0	0.01 \pm 0.0	0.00 \pm 0.0	0.00 \pm 0.0	0.01	**	NS
22:5 ω 3 DPA	0.13 \pm 0.0	0.14 \pm 0.0	0.12 \pm 0.0	0.11 \pm 0.0	0.03	*	NS
22:0	0.05 \pm 0.0	0.05 \pm 0.0	0.06 \pm 0.0	0.05 \pm 0.0	0.01	*	NS
24:0	0.02 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.01	NS	NS
Σ tSFA	64.94 \pm 1.2 ^a	64.21 \pm 1.4 ^a	59.48 \pm 1.1 ^b	57.33 \pm 1.1 ^b	6.17	***	NS
Σ tMUFA	30.18 \pm 1.0 ^b	30.87 \pm 1.1 ^b	35.27 \pm 1.0 ^a	37.08 \pm 1.1 ^a	5.39	***	NS
Σ tPUFA	4.88 \pm 0.3	4.92 \pm 0.3	5.25 \pm 0.3	5.59 \pm 0.2	1.35	NS	NS
$\Sigma\omega$ -3 PUFA	1.12 \pm 0.1	1.15 \pm 0.1	1.24 \pm 0.1	1.26 \pm 0.1	0.55	NS	NS
$\Sigma\omega$ -6 PUFA	2.37 \pm 0.2	2.38 \pm 0.2	2.48 \pm 0.2	2.65 \pm 0.1	0.72	NS	NS
$\Sigma\omega$ -3LC-PUFA	0.27 \pm 0.0	0.27 \pm 0.0	0.25 \pm 0.0	0.23 \pm 0.0	0.06	NS	NS

^{a-c}Values with different superscript are significantly different; ^d Σ tSFA is the sum of 12:0, 13:0, 14:0, 14:0, 15:0, 15:0, 16:0, 16:0, 17:0, 17:0, 18:0, 18:0, 19:0, 20:0, 20:0, 22:0, 24:0; Σ tMUFA is the sum of 14:1 ω -5c, 15:1 ω -6c, 16:1 ω -9c, 16:1 ω -7c, 16:1 ω -7t, 16:1 ω -5c, 16:1, 17:1 ω -8a, 17:0, 17:1 ω -6c, 18:1 ω -9c, 18:1 ω -7c, 18:1 ω -7t, 18:1 ω -5c, 18:1a, 18:1b, 20:1 ω -11c, 20:1 ω -9c, 20:1 ω -7c, 20:1 ω -5c, 22:1 ω -11c, 22:1 ω -9c, 22:1 ω -7c, 24:1 ω -11c, 24:1 ω -9c, 24:1 ω -7c; Σ tPUFA is the sum of 18:3 ω -6, 18:4 ω -3, 18:2 ω -6, 18:3 ω -3, 18:2CLAa, 18:2CLAb, 20:4 ω -6, 20:5 ω -3, 20:3 ω -6, 20:4 ω -3, 20:2 ω -6, 22:5 ω -6, 22:6 ω -3, 22:4 ω -6, 22:5 ω -3; $\Sigma\omega$ -3 LC-PUFA is the sum of 20:5 ω -3, 20:4 ω -3, 22:6 ω -3, 22:5 ω -3; $\Sigma\omega$ -3 PUFA is the sum of 18:4 ω -3, 18:3 ω -3, 20:4 ω -3, 20:5 ω -3, 22:6 ω -3, 22:5 ω -3; $\Sigma\omega$ -6 is the sum of 15:1 ω -6, 17:1 ω -6, 18:2 ω -6, 18:3 ω -6, 20:4 ω -6, 20:3 ω -6, 20:2 ω -6, 22:5 ω -6, 22:4 ω -6; tSFA= total saturated fatty acids; tMUFA= total monounsaturated fatty acids; tPUFA= total polyunsaturated fatty acids; ω -3 FA= total omega-3 fatty acids; ω -6 FA=total omega-6 fatty acids; ω -3 LC-FA=total omega-3 long chain fatty acids; NS = no significance; * = significant ($P < 0.05$); ** = highly significant ($P < 0.01$); *** = very highly significant ($P < 0.001$); ^eTreatment group= group of cows receiving canola oil; TRT=treatment feed, WK= Week, RMSE = root mean square error.

maximum values which were closely scrutinised for any data entry errors. Subsequently, milk fatty acid composition was analyzed by repeated measures analysis of variance (PROC MIXED) in SAS [24] in which treatment, week of lactation and week of lactation by treatment interactions were fitted as fixed effects and cow, baseline milk values as random

effects. Prior to that, compound symmetry covariance structure, linear, quadratic and cubic contrasts were tested in regression analysis and found to have negligible impacts. Separation between means was conducted using Tukey's pairwise comparison and $P < 0.05$ set as the threshold for significance.

Table 4: Mean Fatty Acid Concentration (\pm SE) (% Total FA) of Milk Samples by Week of Supplementation with CDCO

^d Fatty acid (%)	^e Week of supplementation						RMSE	P values	
	0	2	4	6	7	8		WK	TRT*WK
14:0	12.45 \pm 0.3	14.19 \pm 0.3	14.03 \pm 0.4	15.67 \pm 0.6	13.77 \pm 0.5	14.80 \pm 0.6	1.94	NS	NS
15:0	1.22 \pm 0.0	1.85 \pm 0.1	1.76 \pm 0.1	1.60 \pm 0.1	1.53 \pm 0.1	1.49 \pm 0.1	0.37	NS	NS
16:1	0.25 \pm 0.0	0.35 \pm 0.0	0.36 \pm 0.0	0.33 \pm 0.0	0.35 \pm 0.0	0.35 \pm 0.0	0.07	NS	NS
16:0	26.88 \pm 0.8	28.72 \pm 1.0	29.54 \pm 1.4	31.76 \pm 2.3	32.23 \pm 1.4	33.79 \pm 2.2	0.69	NS	NS
17:0	0.60 \pm 0.0	0.64 \pm 0.0	0.62 \pm 0.0	0.58 \pm 0.0	0.59 \pm 0.0	0.58 \pm 0.0	0.10	NS	NS
18:3 ω 6	0.02 \pm 0.0	0.02 \pm 0.0	0.02 \pm 0.0	0.03 \pm 0.0	0.03 \pm 0.0	0.02 \pm 0.0	0.01	NS	NS
18:4 ω 3	0.02 \pm 0.0	0.05 \pm 0.0	0.03 \pm 0.0	0.03 \pm 0.0	0.05 \pm 0.0	0.05 \pm 0.0	0.04	NS	NS
18:2 ω 6	2.58 \pm 0.1 ^a	2.59 \pm 0.1 ^a	2.73 \pm 0.2 ^a	2.08 \pm 0.2 ^b	1.94 \pm 0.2 ^b	1.41 \pm 0.2 ^c	0.69	***	NS
18:3 ω 3	1.11 \pm 0.0 ^a	1.22 \pm 0.1 ^a	1.10 \pm 0.1 ^a	0.91 \pm 0.1 ^{ab}	0.74 \pm 0.1 ^b	0.32 \pm 0.1 ^c	0.53	***	NS
18:1 ω 9c	19.92 \pm 0.5 ^a	16.41 \pm 0.5 ^{bc}	17.35 \pm 0.7 ^{bc}	16.82 \pm 0.9 ^{bc}	18.41 \pm 0.8 ^{ab}	18.63 \pm 1.0 ^{ab}	2.84	**	NS
18:1 ω 7t	6.55 \pm 0.5 ^{ab}	7.76 \pm 0.6 ^a	6.70 \pm 0.6 ^{ab}	5.35 \pm 0.5 ^{bc}	5.55 \pm 0.5 ^{bc}	4.39 \pm 0.4 ^c	2.14	***	NS
18:0	11.96 \pm 0.4	7.79 \pm 0.3	6.86 \pm 0.4	7.50 \pm 0.7	7.59 \pm 0.6	7.63 \pm 0.6	2.09	NS	NS
18:2CLAa	1.07 \pm 0.0	1.25 \pm 0.1	1.37 \pm 0.1	1.32 \pm 0.1	1.36 \pm 0.1	1.06 \pm 0.1	0.31	NS	NS
18:2CLAb	0.24 \pm 0.0	0.28 \pm 0.0	0.27 \pm 0.0	0.21 \pm 0.0	0.25 \pm 0.0	0.31 \pm 0.0	0.08	NS	NS
19:0	0.06 \pm 0.0	0.05 \pm 0.0	0.05 \pm 0.0	0.05 \pm 0.0	0.04 \pm 0.0	0.05 \pm 0.0	0.02	NS	NS
20:5 ω 3 EPA	0.10 \pm 0.0	0.07 \pm 0.0	0.09 \pm 0.0	0.08 \pm 0.0	0.08 \pm 0.0	0.09 \pm 0.0	0.02	NS	NS
20:3 ω 6	0.08 \pm 0.0	0.07 \pm 0.0	0.08 \pm 0.0	0.07 \pm 0.0	0.08 \pm 0.0	0.08 \pm 0.0	0.02	NS	NS
20:4 ω 3	0.06 \pm 0.0	0.04 \pm 0.0	0.04 \pm 0.0	0.03 \pm 0.0	0.04 \pm 0.0	0.04 \pm 0.0	0.02	NS	NS
20:2 ω 6	0.06 \pm 0.0	0.05 \pm 0.0	0.06 \pm 0.0	0.03 \pm 0.0	0.04 \pm 0.0	0.04 \pm 0.0	0.03	NS	NS
20:0	0.14 \pm 0.0	0.10 \pm 0.0	0.10 \pm 0.0	0.10 \pm 0.0	0.10 \pm 0.0	0.11 \pm 0.0	0.04	NS	NS
22:6 ω 3	0.01 \pm 0.0	0.00 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.00 \pm 0.0	0.01 \pm 0.0	0.01	NS	NS
22:4 ω 6	0.00 \pm 0.0	0.00 \pm 0.0	0.00 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.01	NS	NS
22:5 ω 3 DPA	0.12 \pm 0.0	0.11 \pm 0.0	0.13 \pm 0.0	0.13 \pm 0.0	0.13 \pm 0.0	0.15 \pm 0.0	0.03	NS	NS
22:0	0.06 \pm 0.0	0.05 \pm 0.0	0.05 \pm 0.0	0.05 \pm 0.0	0.05 \pm 0.0	0.06 \pm 0.0	0.01	NS	NS
24:0	0.02 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.02 \pm 0.0	0.01	NS	NS
Σ tSFA	59.43 \pm 1.1 ^a	59.62 \pm 1.4 ^a	59.78 \pm 1.7 ^a	64.03 \pm 1.8 ^a	61.73 \pm 1.7 ^a	64.35 \pm 1.7 ^a	6.17	*	NS
Σ tMUFA	35.02 \pm 0.9	34.55 \pm 1.2	34.19 \pm 1.5	30.94 \pm 1.6	33.44 \pm 1.5	31.97 \pm 1.5	5.39	NS	NS
Σ tPUFA	5.55 \pm 0.2 ^{abc}	5.83 \pm 0.3 ^{ab}	6.03 \pm 0.4 ^a	5.03 \pm 0.3 ^{bc}	4.83 \pm 0.3 ^c	3.68 \pm 0.3 ^d	1.35	***	NS
Σ ω -3 PUFA	1.41 \pm 0.1	1.49 \pm 0.1	1.40 \pm 0.2	1.19 \pm 0.1	1.03 \pm 0.1	0.64 \pm 0.1	0.55	NS	NS
Σ ω -6 PUFA	2.83 \pm 0.1	2.82 \pm 0.2	2.99 \pm 0.2	2.31 \pm 0.2	2.19 \pm 0.2	1.67 \pm 0.2	0.72	NS	NS
Σ ω -3LC-PUFA	0.28 \pm 0.0	0.22 \pm 0.0	0.26 \pm 0.0	0.25 \pm 0.0	0.24 \pm 0.0	0.27 \pm 0.0	0.06	NS	NS

^{a-c}Values with different superscript are significantly different; ^d Σ tSFA is the sum of 12:0, 13:0, i14:0,14:0, i15:0, a15:0,15:0, i16:0, 16:0, i17:0, 17:0, i18:0, 18:0, 19:0, 20:0, 20:0, 22:0, 24:0; Σ tMUFA is the sum of 14:1 ω -5c, 15:1 ω -6c, 16:1 ω -9c, 16:1 ω -7c, 16:1 ω -7t, 16:1 ω -5c, 16:1,17:1 ω -8+a17:0, 17:1 ω -6c, 18:1 ω -9c, 18:1 ω -7c, 18:1 ω -7t, 18:1 ω -5c, 18:1a, 18:1b, 20:1 ω -11c, 20:1 ω -9c, 20:1 ω -7c, 20:1 ω -5c, 22:1 ω -11c, 22:1 ω -9c, 22:1 ω -7c, 24:1 ω -11c, 24:1 ω -9c, 24:1 ω -7c; Σ tPUFA is the sum of 18:3 ω -6, 18:4 ω -3, 18:2 ω -6, 18:3 ω -3, 18:2CLAa, 18:2CLAb, 20:4 ω -6, 20:5 ω -3, 20:3 ω -6, 20:4 ω -3, 20:2 ω -6, 22:5 ω -6, 22:6 ω -3, 22:4 ω -6, 22:5 ω -3; Σ ω -3 LC-PUFA is the sum of 20:5 ω -3, 20:4 ω -3, 22:6 ω -3, 22:5 ω -3; Σ ω -3 PUFA is the sum of 18:4 ω -3, 18:3n-3, 20:4 ω -3, 20:5 ω -3, 22:6 ω -3, 22:5 ω -3; Σ ω -6 is the sum of 15:1 ω -6, 17:1 ω -6, 18:2 ω -6, 18:3 ω -6, 20:4 ω -6, 20:3 ω -6, 20:2 ω -6, 22:5 ω -6, 22:4 ω -6; tSFA= total saturated fatty acids; tMUFA= total monounsaturated fatty acids; tPUFA= total polyunsaturated fatty acids; ω -3 FA= total omega-3 fatty acids; ω -6 FA=total omega-6 fatty acids; ω -3 LC-FA=total omega-3 long chain fatty acids; NS = no significance; * = significant ($P < 0.05$); ** = highly significant ($P < 0.01$); *** = very highly significant ($P < 0.001$); ^eweek of supplementation= weeks when cows were fed with canola oil; TRT=treatment feed, WK= Week, RMSE = root mean square error.

RESULTS

Fatty acid Composition of Feedstuff

Table 2 shows that the high CDCO treatment feed contained higher proportions of 16:0, 18:1 ω 9c, total saturated fatty acids (tSFA) and tMUFA than the control treatment. The control feed in turn, had higher proportions of 16:0, 18:2 ω 6, tSFA and tPUFA, whereas basal feed had greater proportion of 18:3 ω 3, tPUFA and ω -3 PUFA. As expected, the CDCO supplement had higher proportions of 18:1 ω 9c, 18:2, 19:0, 20:3 ω 6,

20:0 and tMUFA, but less 18:2 ω 6, 18:3 ω 3 and tPUFA than both the control and pasture basal diets. The pasture basal diet also had the most ALA (18:3 ω 3) as expected.

Fatty Acid Composition of Milk

Canola oil supplementation level affected ($P < 0.05$) some of the fatty acids (Table 3). Fatty acid profiles of the control and treatment groups were largely similar apart from 14:0, 16:0, 18:1 ω 9c, 18:1 ω 7t, tSFA and tMUFA.

Proportion of 18:1 ω 7t in Milk

It was evident that both level and week of CDCO supplementation were significant sources ($P<0.0002$ and $P<0.0001$) of variation influencing 18:1 ω 7t fatty acid. However, supplementation by week interaction had no significant effect ($P<0.3113$; Table 3 and 4). As the level of CDCO was increased, 18:1 ω 7t fatty acid also concurrently increased in the milk. Cows in the high group produced the greatest 18:1 ω 7t percentage in comparison with the control group (7.5 ± 0.5 vs 5.0 ± 0.4), followed by the medium (6.0 ± 0.3 vs 5.0 ± 0.4) and low groups (5.7 ± 0.5 vs 5.0 ± 0.4), respectively.

Proportion of 18:1 ω 9c in Milk

Level ($P<0.0001$) and week ($P<0.002$) of supplementation of cows with CDCO significantly affected the concentration of 18:1 ω 9c in milk. However, the interaction between treatment and week of supplementation had no significant effect ($P<0.08$). The concentration of 18:1 ω 9c in both the high (19.6 ± 0.6)

and medium (19.6 ± 0.6) treatment groups was similar, but higher than the control (16.1 ± 0.6) and low (16.4 ± 0.5) groups (Table 3).

Proportions of tSFA and tMUFA in Milk

As the level of CDCO increased in the diet, the level of tSFA in the milk significantly decreased ($P<0.0001$). The concentration of tMUFA was also significantly affected ($P<0.0001$) by CDCO supplementation (Table 3). However, week of supplementation and its interaction with treatment were not significant ($P<0.1317$ and $P<0.1702$) sources of variation affecting the concentration of tMUFA (Table 4). The high CDCO treatment group yielded the highest proportion of tMUFAs (37.1 ± 1.1 vs 30.2 ± 1.0) compared to the other groups.

Proportion of tPUFA, ω -3 and ω -6 in Milk

Differences in CDCO content in the treatment groups had no significant effect on ω -3 and ω -6 fatty

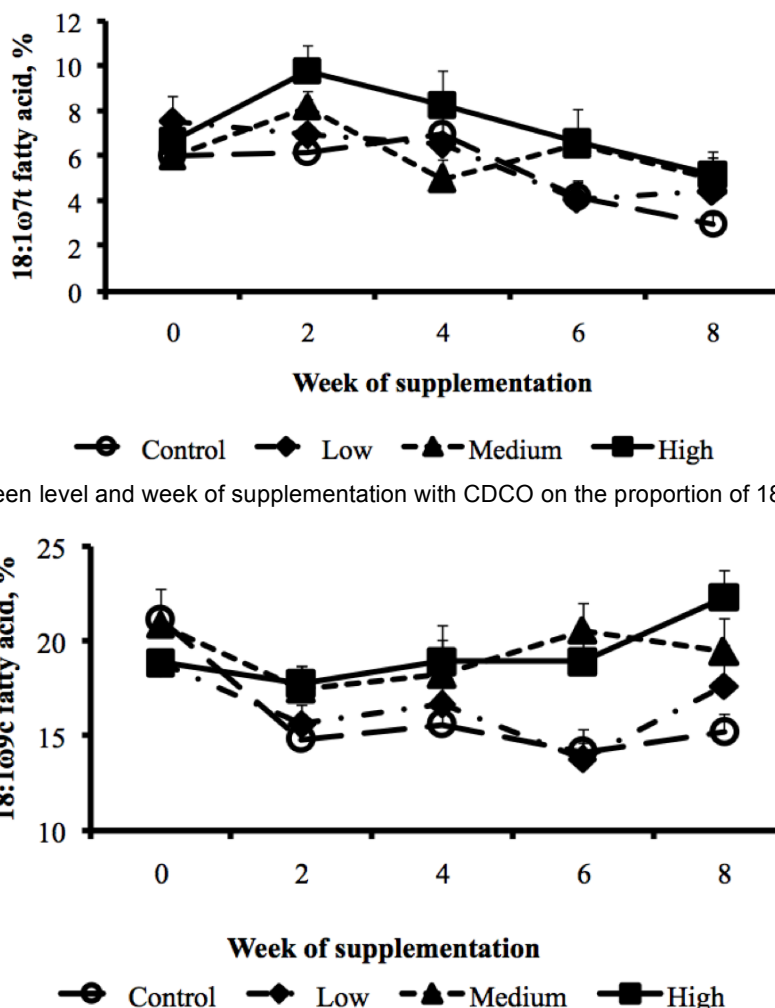


Figure 1: Interaction between level and week of supplementation with CDCO on the proportion of 18:1 ω 7t in milk.

Figure 2: Interaction between level and week of supplementation with CDCO on the proportion of 18:1 ω 9c in milk.

acids. However, week of supplementation was a significant source of variation influencing 18:2 ω 6, 18:3 ω 3, 18:1 ω 9c, 18:1 ω 7t and tPUFA (Table 3 and 4).

Weekly Fatty Acid Composition Trends

As the level of canola oil increased in the diet, weekly 18:1 ω 7t fatty acid also increased (Figure 1). Cows in the high oil treatment group produced the greatest 18:1 ω 7t trend rising from 6.7% to a peak of 9.8% in week two before tapering off to 5.0% at the end of the feeding trial in week eight. Milk 18:1 ω 9c fatty acid concentration increased in the milk of cows receiving medium and high levels of CDCO in the diet (Figure 2). Cows in the control and low treatment groups consistently had the least milk 18:1 ω 9c fatty acid concentration trends throughout the trial period. Cows receiving the high and medium CDCO diets consistently produced milk with lower total tSFA

percentage (Figure 3) compared to the control treatment. However, the cows in the control and low CDCO treatment groups had the greatest weekly tSFA trend. Cows in the high CDCO treatment group had the greatest milk concentration of tMUFA from week two through to eight (Figure 4). tPUFA (Figure 5), ω -6 (Figure 6) and ω -3 (Figure 7) trends were consistently similar for all the treatment groups from weeks zero through to eight.

DISCUSSION

The observed result in the current study where proportions of 18:1 ω 7t increased with incremental levels of CDCO is in agreement with previous studies in dairy cows that utilized canola seed, extruded linseed, and oils from rapeseed, soybean and canola as dietary supplements [25, 26, 27, 15]. The observed

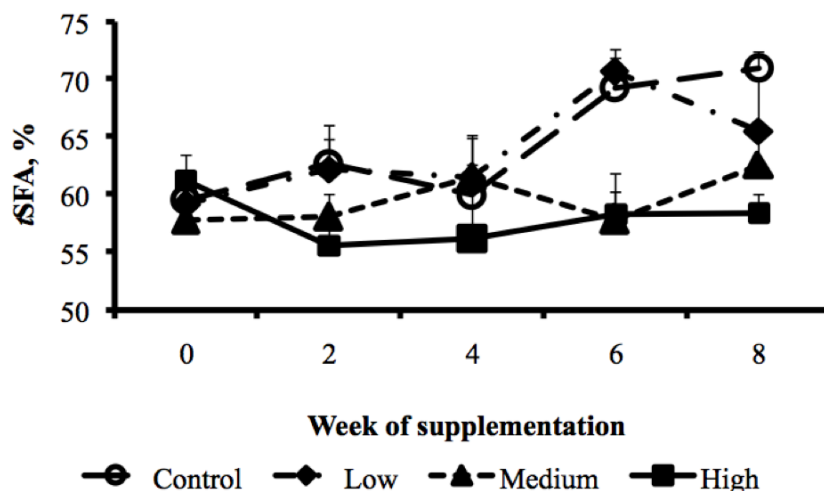


Figure 3: Interaction between level and week of supplementation with CDCO on the proportion of total saturated fatty acid (tSFA) in milk.

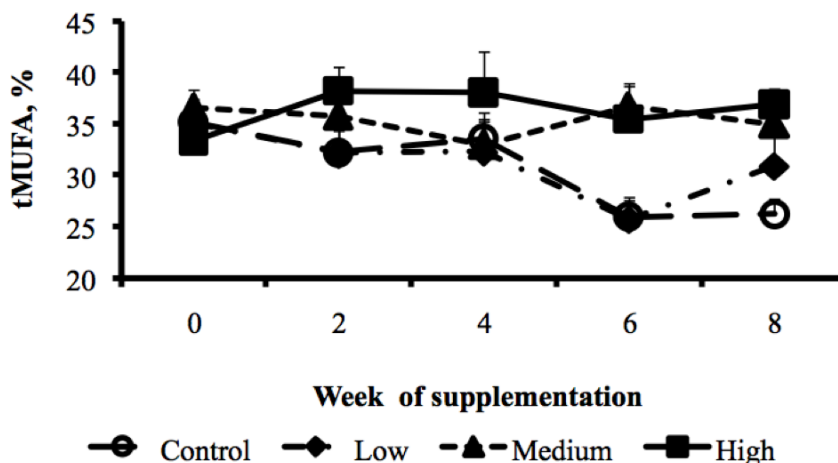


Figure 4: Influence of level and week of CDCO supplementation on the proportion of total monounsaturated fatty acid (tMUFA) in milk.

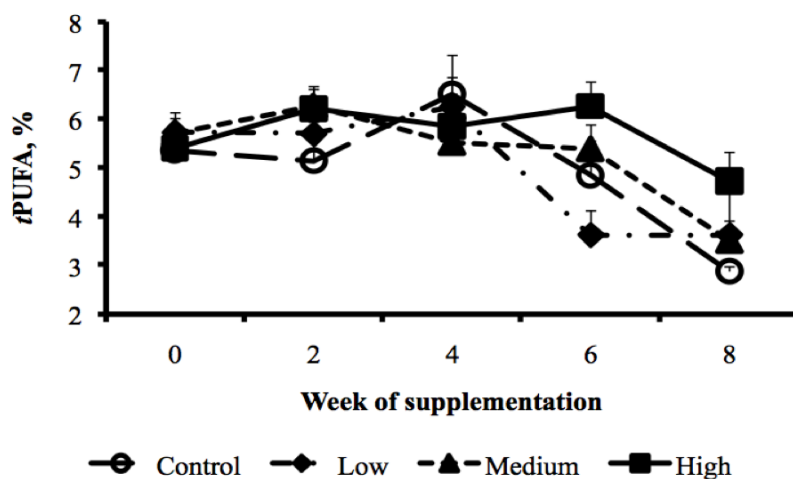


Figure 5: Influence of level and week of supplementation with CDCO on the proportion of total polyunsaturated fatty acid (tPUFA) in milk.

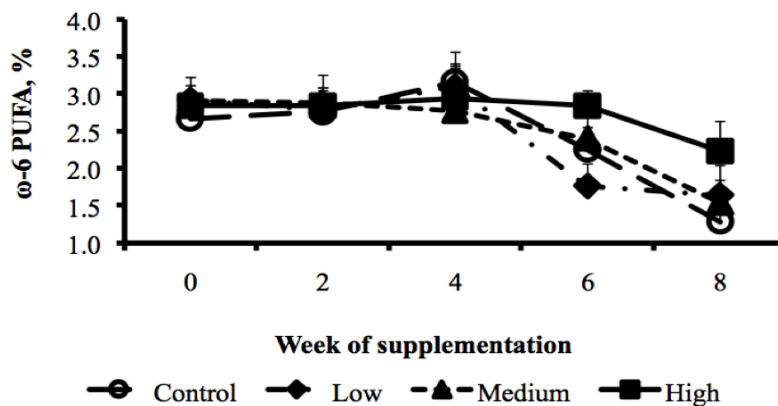


Figure 6: Influence of level and week of supplementation with CDCO on the proportion of ω-6 fatty acids in milk.

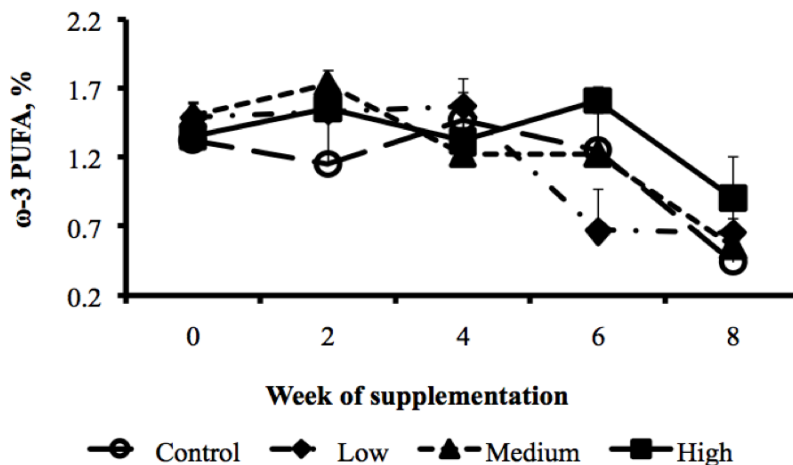


Figure 7: Interaction between level and week of supplementation with CDCO on the proportion of ω-3 fatty acids in milk.

differences in the proportions of 18:1ω7t across treatment groups was possibly due to differences in the proportion of 18:1ω9c between the diets. This is in tandem with the findings of AbuGhazaleh *et al.* [28], that enhancing *trans*-18:1 fatty acid in milk is possible if

a high concentration of dietary 18:1ω9c is available. In this current study, there were high 18:1ω9c proportions in the experimental diets that led to the observed variations in 18:1ω7t in the milk.

Supplementation of lactating cows with CDCO had a positive impact on the proportion of 18:1 ω 9c in agreement with previous studies [2,14,29]. Increased concentration of 18:1 ω 9c is usually associated with rumen biohydrogenation of 18:0, an essential precursor for the synthesis of 18:1 ω 9c [3, 14,30,31,32]. It has also been reported that the majority of 18:1 ω 9c fatty acid found in milk is as a result of desaturation of 18:0 fatty acids in the mammary gland [33]. Previous studies have also indicated that using rich vegetable sources of oleic acid is essential for enhancing the concentration of 18:1 ω 9c in milk fat [34,35,36].

The decrease in the concentration of *t*SFA is consistent with the known effect of canola/rapeseed on milk SFA profile [2,37]. Production of acetic, propionic, and butyric acids by rumen microbes as substrates for energy synthesis have been associated with the production of short and medium branched-chain SFA [38,39]. Therefore, the variation of *t*SFA between groups suggests that addition of CDCO in the diet of lactating cows possibly affected the activities of rumen microbes leading to milk fat depression.

The proportion of milk *t*MUFA was high for cows in the high and medium CDCO treatment groups. This enhanced *t*MUFA is largely due to the elevated 18:1 ω 9c in the diet [14], which aligns with results of previous studies [2,27]. The increasing level of *t*MUFA at the expense of *t*SFA observed in the current study could be beneficial to human health [40]. No significant treatment differences were observed in the proportions of *t*PUFAs, ω -3 and ω -6 fatty acids, while 18:1 ω 7t and 18:1 ω 9c were significantly increased by the duration of CDCO supplementation. This lends credence to the report of Martínez-Marín *et al.*, [41] who demonstrated that in goats, time is an important factor in the modification of milk fatty acids. Therefore, the duration of supplementation may be just as crucial as the dietary composition in modifying milk fatty acid composition in grazing cows.

Therefore, the current results seem to suggest that to enhance the proportions of 18:1 ω 9c, 18:1 ω 7t and *t*MUFA at the expense of *t*SFA, primiparous Holstein-Friesian dairy cows grazing pastures need to be supplemented with CDCO at levels greater than 35ml /Kg DM for a duration of eight weeks and *t*MUFA will continue to rise linearly at the expense of *t*SFA (Figures 3 and 4). Our current study also did agree with the findings of Ferlay *et al.*, [27] who found that feeding

linseed to dairy cows increased the proportion of MUFA at the expense of SFA.

CONCLUSIONS

The observed increases in the proportions of 18:1 ω 9c, 18:1 ω 7t and *t*MUFA at the expense of *t*SFA, suggest that the supplementation of grazing primiparous Holstein-Friesian cows with CDCO can potentially improve milk quality and enhance its beneficial healthy fatty acid profile without any negative impact on the animals or milk taste. Therefore, the tested hypothesis that *incremental supplementation of grazing primiparous Holstein-Friesian cows with CDCO will alter milk fatty acid composition towards increased total monounsaturates* holds true and should be accepted.

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